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Potential of Effective Microorganisms in the Aspect of Sustainable Development

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Abstract: Human activity has been impacting the environment for thousands of years. Progressing environmental pollution caused by human economic activity is associated with an increase in the concentration of trace elements and the formation of an excess of polycyclic aromatic hydrocarbons (PAHs). PAHs have always been present in the environment, but the development of civilisation and technology has resulted in their increase. PAHs easily penetrate plants, animals and the human body, which makes them a serious threat to human health and agriculture. Reducing the amount of polycyclic aromatic hydrocarbons in the soil is possible by introducing a microbiological preparation in the form of Effective Microorganisms (EM) into the soil. Some strains of microorganisms in EM can decompose chemical compounds, including PAHs. Through biodegradation, EM transforms harmful substances into less toxic forms. EM stimulates the activity of soil microflora, which naturally participates in the decomposition processes of organic substances, which contributes to improving soil quality and environmental protection. The following work presents the results of own research on analysing the impact of Effective Microorganisms on changes in the content of PAHs in the soil. It was observed that 75% of test samples in the form of soils after wheat cultivation in which EM was added had lower contents of 3,4,5-ring PAHs than their control samples. The work aims to analyse the potential of EM as a preparation that supports the natural capabilities of the ecosystem to reduce the amount of pollutants in the form of PAHs in the soil.

Keywords: polycyclic aromatic hydrocarbons, circular economy, agriculture, Effective Microorganisms

1. Introduction

Europe is facing potentially irreversible climate change, environmental degradation and the resulting loss of biodiversity, and rising levels of waste and pollution. It could pose an increasing threat to citizens' economic and social well-being. Given the above, in-depth research into new concepts and forms of sustainable development can be observed for all sectors of the economy, including the agri-food sector (Gralak 2021, Gralak et al. 2022, Kaim 2021).

Progressive degradation and pollution of the natural environment are consequences of the development of civilisation, intensive farming, overexploitation of raw materials and population pressure. The need to protect and restore ecosystems, revitalise anthropogenically damaged areas, and eliminate or neutralise chemical contamination is a huge challenge for modern science (Bradshaw et al. 2021, Kaszycki et al. 2022).

Land and water are natural resources and constitute a very important source of maintaining agricultural production, which has increased significantly in recent decades. This growth has made agriculture the largest and most important economic sector in many countries, creating the most employment opportunities in the world (Duque-Acevedo et al. 2020, FAO 2019, Khan et al. 2021). This "green revolution" has changed the world of agriculture, increasing the use of agricultural products, maximising crop yields, and thus overcoming the food crisis. Development of the agricultural sector also has a disturbing impact on the environment (Aznar-Sánchez 2018, Aznar-Sánchez 2019, Lange et al. 2018).

Changing the environment to meet social needs can have serious consequences. Environmental pollution caused by human economic activities is associated with an increase in the concentration of trace elements but also with the formation of excessive polycyclic aromatic hydrocarbons. PAHs are highly stable in the environment and bioaccumulate in soil and aquatic sediments. Due to their hydrophobicity and stable chemical structure, PAHs quickly accumulate in the organic components of the soil. PAHs are natural compounds that occur in various structural forms. They are essential for the environment due to their strong genotoxic, mutagenic and carcinogenic properties. A few studies focus only on the content of these compounds on the soil surface (Cornflower & Gworek 2020, Respondek et al. 2022, Sawicka 2019).

Surface soils are large reservoirs that absorb PAHs due to their physicochemical properties, allowing these compounds to adsorb onto soil particles. PAHs are toxic compounds with ecotoxic, genotoxic and mutagenic properties, which have been confirmed in several studies, including by the Institute of Cancer Research (ILCR).

One potential solution to ensuring environmental stability is using effective microbial (EM) forms of biological agents, initially developed by Professor Teruo Higa of Ryukyus University in Okinawa. EMs are beneficial to the environment. They are responsible for the processes of restoration, reconstruction and renewal. EM is a mixture of microorganisms that can coexist and positively influence soil properties and plant growth (Armand et al. 2021, Piotrowska & Boruszko 2022, Terech-Majewska et al. 2021).

The microorganisms in EM formulations are highly adaptive. They form a two-layer capsule with an aerobic environment on the outside and an anaerobic environment on the inside. The zone separating the two environments are facultative organisms, which carry out aerobic metabolism on the outside and anaerobic metabolism on the inside. They have tremendous biological potential due to specialised enzymes that can convert troublesome compounds into useful forms (Auriga 2021, Dobrzyński et al. 2021, Kaboosi et al. 2022).

2. Materials and Methods

The research was conducted at the Chemical Laboratory of the Faculty of Civil Engineering and Environmental Sciences of the Bialystok University of Technology. Gas chromatography-mass spectrometry (GC-MS) was used to quantify the content of polycyclic aromatic hydrocarbons in soil. The tests were carried out following the PN-ISO 18287:2008 standard. Soil material from the pot experiment was used for the research, i.e. soil before sowing the plants and soil two weeks after the experiment. Two plants were selected to study changes in PAH content: one from a monocotyledonous plant, wheat (*Triticum aestivum L.),* and the other from a dicotyledonous plant, rapeseed (*Brassica napus L.*). The selection of plants was based on their belonging to various systematic classes, following the recommendations of the ISO 11269 (1995) standard. These plants were also used in previous research at the Institute of Fertilization and Soil Science in Puławy.

The PAH contents were determined in four different types of soil:

- The first type is high-quality potting soil from COMPO SANA. According to the manufacturer, it is the highest quality, universal, perfectly composed substrate with a pH of 5.0-6.5 for planting all plants grown at home, on the balcony, terrace or garden. The exclusive recipe is based on selected peat fractions and contains enriching additives that positively affect plant development. The starting dose of fertiliser provides access to nutrients for up to 8 weeks after planting the plant. Volcanic perlite is intended to loosen the substrate, ensuring air, water and nutrients access to the roots.
- The second type is universal soil produced by AB. According to the manufacturer, it is a ready-made substrate for growing plants with average nutritional requirements with a pH of 5.5-6.5. Perfect for planting potted plants and for use in flower gardens. It is distinguished by its excellent structure, which ensures good air and water permeability, thus ensuring proper rooting of plants. Thanks to its properties, it provides appropriate conditions for their optimal vegetation, which results in beautiful flowering.
- The third type is a horticultural substrate for sowing and quilting produced by Athena. According to the manufacturer, it is intended for pruning plants, replanting them and sowing seeds. The mixture with a pH of 5.0-6.5 contains peat, lime and fertiliser to provide the plants with the necessary nutrients. The composition has been composed to provide young plants with optimal conditions for growth. A product with a fine structure promotes rooting. The gardening soil for sowing and planting is rich in minerals, guaranteeing rapid plant growth.
- The fourth type is acidic peat produced by Athena. According to the manufacturer, acidic peat with a pH of 3.5-4.5 will fertilise the soil and acidify it, providing acid-loving plants with optimal conditions for growth. The product stores water very well. Thanks to this, the vegetation will be protected from drying out. Acidic peat is rich in humus, provides good drainage and protects the substrate from drying out and silting up.

The content of 16 PAHs was determined in μg/kg DM such as:

- 2-r – naphthalene;
- 3-r – acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene;
- 4-r – fluoranthene, pyrene, benzo[a]anthracene, chrysene;
- 5-r – benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene;
- 6-r – indeno [1,2,3,c,d]pyrene, benzo[g,h,i]perylene.

Extraction was carried out using acetone and petroleum ether. Acetone is an effective extractant mainly because it enables the breakdown of soil aggregates. Petroleum ether increases extraction efficiency and is necessary as a solvent in the subsequent concentration procedure (Haleyur et al. 2016). A soil sample with field moisture content was extracted twice.

In the first step, 25 g of soil was weighed and placed in a conical flask. Then, 50 ml of acetone was added and extracted by shaking for 1 h in a shaker. In the next step, 50 ml of petroleum ether was added, shaken again, then decanted and shaken with another 50 ml of petroleum ether. After settling the soil particles, the liquid from above the precipitate was decanted. The extracts were combined, and acetone and other polar compounds were removed by shaking twice with 400 ml of water. The water was discarded. The resulting organic layer was dried using anhydrous sodium sulfate. The extract was then analysed using capillary gas chromatography. Quantification of PAH content was carried out by mass spectrometry detection, using the corresponding deuterated PAHs as internal standards.

The experimental system covering soil after wheat cultivation:

- P1 – universal substrate of the highest quality, on which wheat grew for 2 weeks, with distilled water;
- P2 – universal substrate of the highest quality, on which wheat grew for 2 weeks, with 1% EM solution;
- P3 – universal soil in which wheat grew for 2 weeks, with distilled water;
- P4 – universal soil in which wheat grew for 2 weeks, with a 1% EM solution;
- P5 – horticultural substrate for sowing and quilting, on which wheat grew for 2 weeks, with distilled water;
- P6 – horticultural substrate for sowing and planting, on which wheat grew for 2 weeks, with a 1% EM solution;
- P7 – acidic peat substrate on which wheat grew for 2 weeks, with distilled water;
- P8 – acidic peat substrate on which wheat grew for 2 weeks, with a 1% EM solution.

The experimental system covering soil after rapeseed cultivation:

- R1 – universal substrate of the highest quality, on which rapeseed grew for 2 weeks, with distilled water;
- R2 – universal substrate of the highest quality, on which rapeseed grew for 2 weeks, with 1% EM solution;
- R3 – universal soil on which rapeseed grew for 2 weeks, with distilled water;
- R4 – universal soil on which rapeseed grew for 2 weeks, with a 1% EM solution;
- R5 – gardening substrate for sowing and planting, on which rapeseed grew for 2 weeks, with distilled water;
- R6 – horticultural substrate for sowing and planting, on which rapeseed grew for 2 weeks, with a 1% EM solution;
- R7 – acidic peat substrate on which rapeseed has not grown for 2 weeks, with distilled water;
- R8 – acidic peat substrate on which rapeseed has not grown for 2 weeks, with a 1% EM solution.

The experimental system, including soil before cultivation:

- G1 – universal substrate of the highest quality before cultivation;
- G2 – universal soil before cultivation;
- G3 – horticultural substrate for seeding and quilting before cultivation;
- G4 – acid peat substrate before cultivation.

3. Results and Discussion

The results of gas chromatography analysis with mass spectrometry (GC-MS) detection are shown in Figures 1, 2 and 4. The content of polycyclic aromatic hydrocarbons in soils was determined by soil type, EM watering and plant type. The highest contents of polycyclic aromatic hydrocarbons were recorded in the soil before cultivation for the highest quality universal soil, universal soil and horticultural sowing and quilting medium.

The highest content of 16 PAHs of 296.59 µg/kg DM was in G4 soil (acid peat substrate before cultivation) relative to the other pre-till soils. The lowest content of the total 16 PAHs of 143.55 µg/kg DM was characterised by soil G2 (universal soil before cultivation) also concerning the other pre-till soils. Pre-till soils in the form of G1 (universal soil of the highest quality before cultivation) and G3 (horticultural soil for seeding and quilting before cultivation) were characterised by a similar sum of 16 PAHs amounting to 195.34 µg/kg DM and 199.6 µg/kg DM, respectively.

Numerous studies presented in the literature on the problem of soil pollution have shown that hydromorphic soils, even unpolluted ones, can contain elevated amounts of PAHs reaching 200-249.6 µg/kg DM in the 0-5 cm layer. Analysing literature data on PAH content, the authors show that land use significantly affects topsoil. Urban soils exposed to traffic and industrial pollution contain 5-10 times more PAHs than agriculturally used soils (Ciesielczuk 2021, Kumar et al. 2021, Yakovleva et al. 2022).

Based on the current Regulation of the Minister of the Environment, dated September 1, 2016, on assessing land surface contamination, naftelen is classified as a substance causing risks of particular relevance to land surface protection. Its permissible content in soil and permissible content in soil for subgroup II is 0.1 mg/kg DM (Minister of the Environment 2016). According to the analysis results, the naphthalene content in samples G1, G2, G3 and G4 ranges from 0.07 mg/kg DM to 0.11 mg/kg DM, which is acceptable by the standards. However, you'll notice that the naphthalene content is close to the acceptable upper limit. If the upper limit of acceptable naphthalene content is critical from the point of view of environmental standards, steps should be taken to minimise this content.

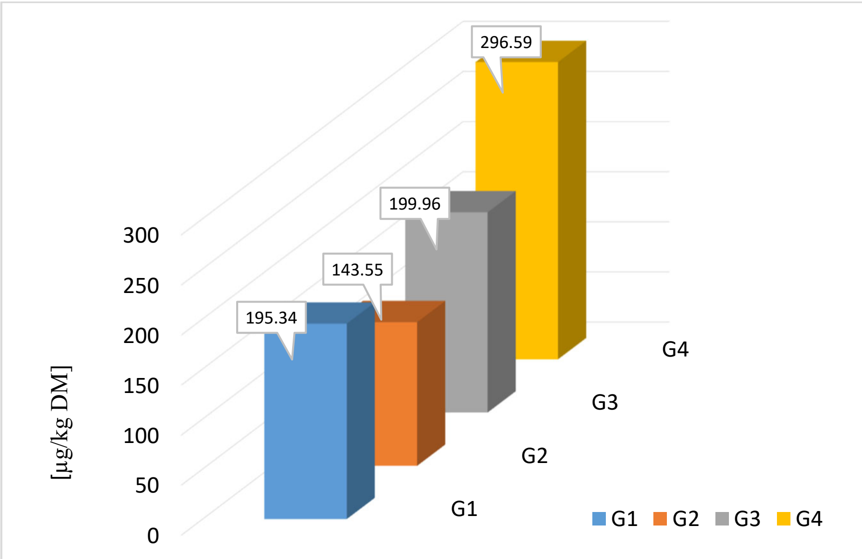

Fig. 1. Content of sum of 16 PAHs in soils before crop cultivation

Total concentrations of sixteen PAHs were higher than the test samples for 3 of the 4 control soil samples on which watered wheat was grown. Lower concentrations of PAHs were exhibited by research samples P4 (universal soil in which wheat grew for 2 weeks, with a 1% EM solution), P6 (horticultural substrate for sowing and planting, on which wheat grew for 2 weeks, with a 1% EM solution), P8 (acidic peat substrate on which wheat grew for 2 weeks, with a 1% EM solution) concerning their control samples.

Analysis of the results allows us to conclude that there were fewer 2-ring PAHs in all the research samples in the form of wheat-grown soils concerning their control samples. It was observed that 75% of the research samples, in the form of post-wheat soils, had lower contents of 3,4,5-ring PAHs than their control samples. Slight changes in content between the control samples in the form of post-wheat soils and the research samples were observed in 6-ring PAHs.

Naphthalene was the dominant hydrocarbon in the post-wheat soils. Acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene were present in relatively high concentrations in the control sample P7 (acidic peat substrate on which wheat grew for 2 weeks, with distilled water) in relation to its research sample P8. Significant decreases in the concentrations of fluoranthene, pyrene, benzo[a]anthracene and chrysene were observed in research sample P6 concerning its control sample P5 (horticultural substrate for sowing and quilting, on which wheat grew for 2 weeks, with distilled water) and between research sample P8 and its control sample P7. Indeno [1,2,3,c,d]pyrene, and benzo[g,h,i]perylene were present in relatively low concentrations.

The decisive influence on the sum content of PAHs in soils after wheat cultivation was 2-ring PAHs. Naphthalene concentration was highest in the P7 control sample compared to other compounds. In the control samples, naphthalene concentrations were maintained in the range of 20.66 to 67.07 µg/kg DM, which accounted for 46.3-52.2% of the total concentration of the 16 labelled PAHs among the control samples. In the research samples, naphthalene concentrations maintained a range of 17.72 to 45.22 µg/kg DM, which accounted for 52.8-60.9% of the sum concentration of the 16 labelled PAHs among the research samples.

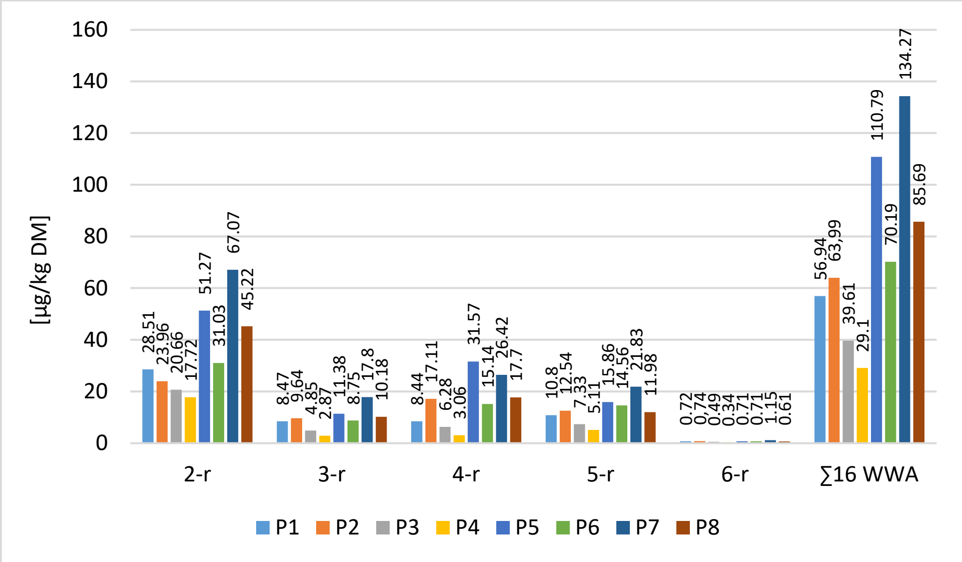

Fig. 2. PAH concentrations in soils after wheat cultivation

Figure 3 shows a comparison of the percentage decrease in the content of 2,3,4,5,6-ring PAHs of the sample with acid peat on which 2 weeks of wheat was grown watered with a 1% solution of EM Naturally Active to the sample with acid peat on which 2 weeks of wheat was grown watered with distilled water.

There was the greatest decrease in the content of 2,4-ring PAHs in the P8 trial relative to P7. The naphthalene content decreased by 67.4% in the P8 test sample with respect to its P7 control sample. According to literature data, low-molecular-weight PAHs (2 and 3 rings) volatilise, leach or degrade more easily than high-molecular-weight PAHs (Soukarieh et al. 2018).

A significant decrease from the P8 sample to P7 was also reported for 4-ring PAHs, which include fluoranthene, pyrene, benzo[a]anthracene and chrysene, amounting to 66.0%.

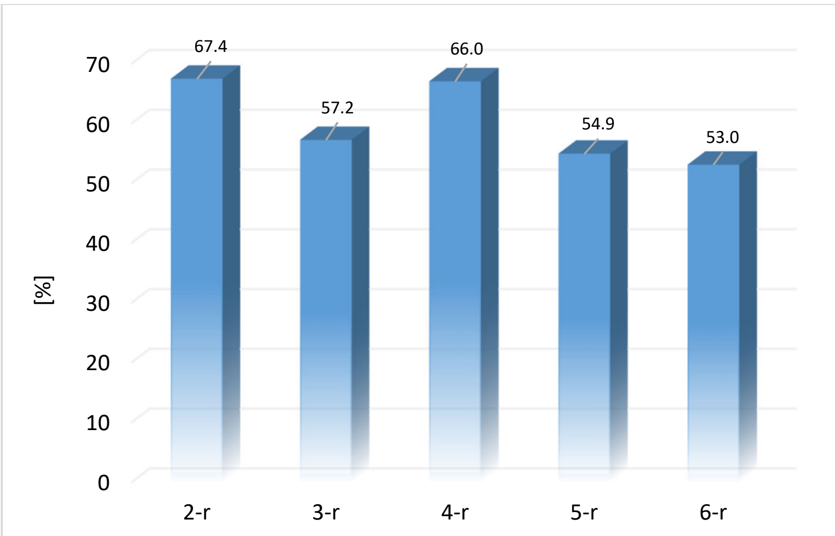

Fig. 3. Comparison of the percentage decrease in PAH content of test sample P8 to control sample P7 (100%) in medium with acid peat

EM-watered soils on which rapeseed grew had higher PAH content than water-only soils on which rapeseed grew. It represents a different relationship than in the case of soil after wheat cultivation.

In the samples in which acid peat was used as a substrate for rapeseed cultivation, the highest PAH content was observed in research sample R8 (acidic peat substrate on which rapeseed has not grown for 2 weeks, with a 1% EM solution).

Concentrations of 2-ring compounds in soils after oilseed rape cultivation varied, taking values in control samples in the range of 34.03 to 53.93 µg/kg DM and in research samples in the range of 52.39 to 95.87 µg/kg DM.

The results indicate that the concentrations of 3-ring PAHs after rapeseed cultivation remained similar to those of 5-ring PAHs. The concentrations of 3-ring PAHs ranged from 8.25 to 15.68 µg/kg DM in the control samples and 9.9 to 26.51 µg/kg DM in the research samples. The concentrations of 5-ring PAHs ranged from 9.03 to 13.3 µg/kg DM in the control samples and 12.5 to 31.06 µg/kg DM in the research samples.

Six-ring PAHs had the least significant effect on total PAH content in soils after rapeseed cultivation. Concentrations were lowest of indeno [1,2,3,c,d]pyrene and benzo[g,h,i]perylene in control sample R4 (all-purpose soil on which rapeseed was grown for 2 weeks watered with distilled water 1% solution of EM Naturally Active) compared to other compounds.

In the control samples after rapeseed cultivation, the concentrations of indeno [1,2,3,c,d]pyrene and benzo[g,h,i]perylene were maintained in the range of 0.86 to 2.27 µg/kg DM, which was 1.1-2% of the total concentration of the 16 labelled PAHs among the control samples. In the research samples, the concentrations of indeno [1,2,3,c,d]pyrene and benzo[g,h,i]perylene were maintained in the range of 0.76 to 2.85 µg/kg DM, which accounted for 0.8-1.2% of the sum concentration of the 16 labelled PAHs among the research samples.

It was observed that whether after rapeseed or wheat cultivation, 2-ring PAHs had the greatest impact on the summed PAH content of soils. Soil treated with EM after wheat cultivation contained 2-ring PAHs up to 17.72 to 45.22 µg/kg DM, while soil treated with EM after rapeseed cultivation contained significantly more 2-ring PAHs, i.e. 52.39 to 95.97 µg/kg DM.

Control samples in the form of soil after wheat cultivation had total concentrations of 16 labelled PAHs ranging from 39.61 to 134.72 µg/kg DM, and the control samples after rapeseed cultivation had summed concentrations of 16 labelled PAHs from 81.63 to 115.76 µg/kg DM. The test samples in the form of soil after wheat cultivation had summed concentrations of 16 labelled PAHs from 29.1 to 85.69 µg/kg DM, and the test samples after rapeseed cultivation had summed concentrations of 16 labelled PAHs from 95.02 to 240.46 µg/kg DM.

PAHs are generally considered insoluble in water (and therefore unavailable to plants), and the solubility of these compounds (despite their hydrophobic nature) is relatively high. However, due to the co-occurrence of natural surfactant-like compounds and the action of soil fauna enzymes, a small amount of them can pass into a water-soluble form. This indicates that plants can take them up and accumulate in tissues, which may pose a risk due to carcinogenic and mutagenic effects (Ciesielczuk 2021, Gacek 2021).

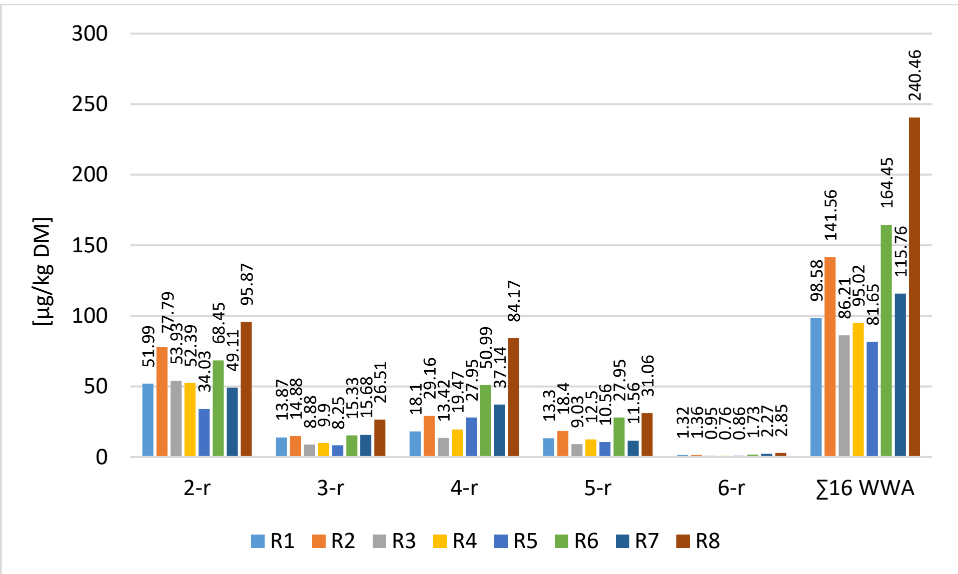

Fig. 4. PAH content in soils after rapeseed cultivation

Figure 5 shows the changes in the percentage of 3,4,5,6-ring PAHs in relation to the sums of 16 PAHs (100%) determined in soils after wheat and rapeseed cultivation. Analysis of the results allows us to conclude that the percentage of 4-ring PAHs in the soil after rapeseed cultivation was greater than the percentage after wheat cultivation in relation to the sums of 16 PAHs.

An inverse relationship was observed for 5-ring PAHs, the percentages of which were higher in soil after rapeseed and lower in soil after wheat. Changes in the percentage of 3,6-ring PAHs were similar in both soil after wheat and rapeseed.

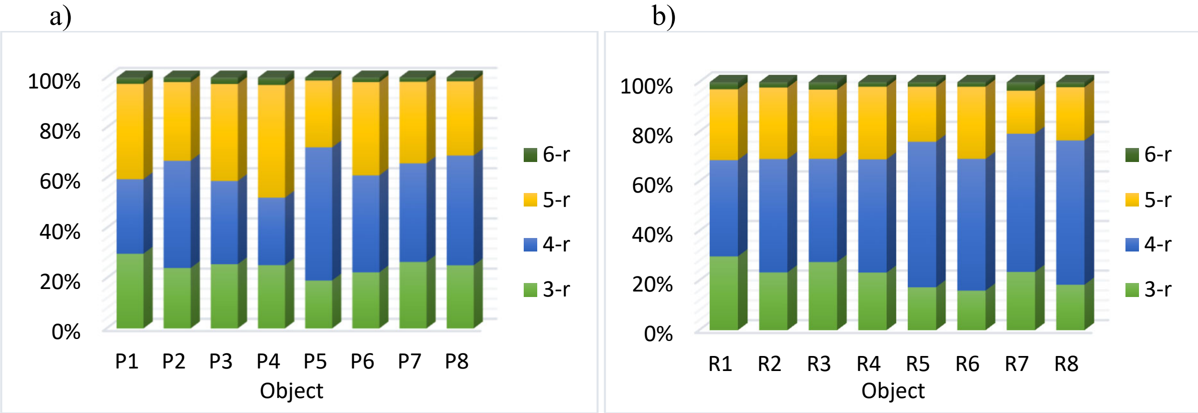

Fig. 5. Changes in the percentage of 3,4,5,6-ring PAHs in relation to the sum of 16 PAHs (100%) determined in soils after growing a) wheat, b) rapeseed

According to many study authors, PAHs can be decomposed using microorganisms. It involves the decomposition of organic compounds through biotransformation to less complex metabolites and through mineralisation to inorganic minerals such as H_2O, CO_2 (aerobic) or CH_4 (anaerobic) (Abdel-Shafy & Mansour 2016, Mackiewicz-Walec & Krzebietke 2020, Nguyen et al. 2020).

Among PAH remediation methods, biological treatment based on microbial transformation and degradation has received considerable attention. In the literature, we can find information that biological treatment is much more effective for degrading low molecular weight PAHs (Wang et al. 2019, Dai et al. 2022).

4. Conclusions

Tackling soil contamination, including PAH contamination, belongs to activities based on closed-loop economy models. The problem of land contamination with organic substances in the form of PAHs is an important issue due to the negative environmental impact of this type of pollution. The harmfulness of organic PAHs is due to their chemical and physical properties, which disturb the biological balance in the soil.

Organic fertilisers in the form of EM are produced using living microorganisms, delivering nutrients to the soil and allowing inactive ones to become available. The study showed that the content of Polycyclic Aromatic Hydrocarbons in soils was determined by soil type, EM watering and plant type. In addition, the soils on which wheat was grown showed a higher content of 16 PAHs in 75% of the control samples compared to the sum of 16 PAHs in the research samples watered with EM.

The experiment results indicate that naphthalene is the main hydrocarbon in the soil after wheat cultivation. The greatest decrease in the content of 2,4-ring PAHs was shown by trial P8 (acidic peat substrate on which wheat grew for 2 weeks, with a 1% EM solution) in relation to P7 (acidic peat substrate on which wheat grew for 2 weeks, with distilled water). Naphthalene content decreased by 67.4% in the P8 test sample with respect to its P7 control sample.

The concentrations of acenaphthene, acenaphthene, fluorene, phenanthrene, and anthracene were relatively higher in the P7 control sample than in the P8 research sample. Analysis of the results shows that after rapeseed and wheat cultivation, 2-ring PAHs had the greatest impact on the content of 16 PAHs in soils.

Based on the analysis of the collected data, it can be concluded that applying the EM preparation on the soils with wheat significantly reduced the amount of PAH pollutants in the soil. It provides an opportunity to accelerate the decomposition of organic pollutants in the soil without moving it and using physical neutralisation methods.

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